IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

APPLICANT: Brod, S.	§	**ART UNIT: 1761**
FILED: April 12, 1997	§	
	§	**EXAMINER:**
SERIAL NO.: 08/631,470	§	**Sayala, C.**
	§	
FOR: Methods of Treating Autoimmune Diseases Using Type One Interferons	§	**DOCKET: D5716CIP2**

The Assistant Commissioner of Patents and Trademarks
BOX AF
Washington, DC 20231

ATTENTION: Board of Patent Appeals and Interferences

APPELLANT'S BRIEF

This Brief is in furtherance of the Notice of Appeal filed in this case on December 16, 1998. The fees required under 37 C.F.R. §1.17(f) and any other required fees are dealt with in the accompanying TRANSMITTAL OF APPEAL BRIEF.

In accordance with 37 C.F.R. §1.192(a), this Brief is submitted in triplicate.

INDEX OF SUBJECT MATTER

I. REAL PARTY IN INTEREST

The real party in interest is the Research Development Foundation, the Assignee, as evidenced by Assignments recorded in the Patent & Trademark Office at Reel 8004, frame 0638, on June 17, 1996, and at Reel 7014, frame 0536, on May 27, 1994.

II. STATUS OF THE CLAIMS

Originally, claims 1-20 were filed with this Application. Claims 1, 8, and 19 have been amended. The pending claims 1-20 are being appealed, of which claims, 1, 8, 13, and 19 are independent claims.

III. STATUS OF AMENDMENTS

Claims 1, 8, and 19 were amended in the Response to Office Action filed October 7, 1997. All pending claims are shown in Appendix A.

IV. STATEMENT OF RELATED APPEALS AND INTERFERENCES

To Appellant's knowledge, the pending appeal of related applications Serial Nos. 08/631,470 and 08/946,710 may directly affect or be directly affected by the present appeal.

V. SUMMARY OF THE INVENTION

The present invention describes the oral administration of a type one interferon in an animal (page 72, lines 4-6), particularly for the treatment of the autoimmune diseases of in humans such as multiple sclerosis (page 16, lines 10-14). Oral administration of type one interferon is also used for the reduction of inflammation associated with an auto-immune disease (page 72, lines 6-9). Generally, the preferred interferon in the methods of the present invention is either alpha-interferon or beta-interferon (Page 16, lines 17-19). A wide variety of auto-immune diseases may be treated by the methods of the present invention (page 17, lines 6-7). Representative examples of auto-immune diseases include multiple sclerosis, rheumatoid arthritis, diabetes mellitus, psoriasis, organ-

specific auto-immune diseases, chronic inflammatory demyelinating polyradiculoneuropathy and Guillain-Barré syndrome (page 17, lines 7-11).

Oral administering a type one can also be used to reduce the levels of cytokines including TGF-β, IL-2, IL-10, IFN-γ and the inflammatory soluble serum marker ICAM-1 in an individual having multiple sclerosis, (page 72, lines 9-14). Parenterally administered human recombinant type I interferons [hrIFN] in relapsing-remitting multiple sclerosis have been demonstrated to decrease relapses and spontaneous *in vitro* IFN-γ production, reduce clinical progression, and decrease magnetic resonance imaging-defined disease activity and lesions (page 5, lines 19-23). The present invention shows that ingested human IFN-α is also a profound biological response modifier in humans (Page 6, lines 14-16).

VI. ISSUES

A. 35 U.S.C. §102

(1) Whether claims 1-4, 6-11, and 13-20 are anticipated by **Cummins, Jr.** (U.S. Patent 5,019,382) under 35 U.S.C. §102(b).

B. 35 U.S.C. §103

(1) Whether claims 5 and 12 are obvious over **Cummins, Jr.** (U.S. Patent 5,019,382) under 35 U.S.C. §103.

(2) Whether claims 1-20 are obvious over by **Cummins, Jr.** (U.S. Patent 5,019,382) in view of **Shibutani** et al. (Iyakuhin Kenkyu, vol. . 18(4), pp. 571-82, 1987) under 35 U.S.C. §103.

C 35 U.S.C. §112

(1) Whether claims 1-12 and 19-20 are not enabled under 35 U.S.C. §112 because there is no support in the specification for the terms "immediately upon" in reference to ingestion after oral administration."

VII. GROUPING OF CLAIMS

The rejected claims do not stand or fall together. The Applicant considers claims 1-20 to lie in four embodiments of the present invention. Claims 1-7 are drawn to a method of treating an auto-immune disease in an animal by ingestion upon oral administration of a type one interferon. Claim 8-12 are drawn to a

method of decreasing the severity or frequency of relapse of multiple sclerosis in a human by oral administration and ingestion of a type one interferon. Claims 13-18 involve the reduction of inflammation associated with an autoimmune disease by oral administration and ingestion of a type one interferon. Claims 19-20 entail administration of a type one interferon by oral administration followed by immediate ingestion to reduce cytokine levels such as TGF-β, IL-2, IL-10, INF-γ, and ICAM-1 in an individual having multiple sclerosis.

VIII. ARGUMENTS

A. 35 U.S.C. §102

(1) The rejection of claims 1-4, 6-11, and 13-20 under 35 U.S.C. §102(b) as anticipated by **Cummins, Jr.** (U.S. Patent 5,019,382) was maintained in the Office Action mailed July 16, 1998. The Examiner maintains that col. 4, lines 19-36; col. 5, lines 50-55; col. 6, lines 12-26; col. 13 and, the claims of **Cummins, Jr.** (U.S. Patent 5,019,382) anticipate claims 1-4, 6-11, and 13-20. The

Examiner claims, "Such disclosure meets the claims." The Applicant respectfully disagrees.

It is well established in United States patent law that an anticipatory reference under 35 U.S.C. §102 must be enabling to the same degree as an invention seeking patent protection under 35 U.S.C. §112, first paragraph. An analysis of **Cummins** reveals deficiencies with regard to enablement which prevent **Cummins** from anticipating the Applicant's invention. The Applicant's position is supported by the two Declarations under 37 C.F.R. 1.132 submitted previously. The Rule 1.132 Declarations of John Lindsey, M.D., Assistant Professor of Neurology, and Jerry S. Wolinsky, M.D., Professor or Neurology. Both Declarants are skilled in the area of autoimmune diseases in general and in multiple sclerosis in particular. The Rule 1.132 Declarations support and expand upon the following arguments of the Applicant.

Within Column 4, lines 19-36 of **Cummins**, the disease conditions are listed to which the **Cummins** patent may be directed: ". . . include apparent autoimmune disorders characterized by a chronic tissue degenerative inflammatory condition. Diseases so characterized include multiple sclerosis, rheumatoid arthritis, stomatitis, and lupus erythematosus." However, only one anecdotal

example is given describing treatment of multiple sclerosis and none for human lupus erythematosus. Most of the diseases actually described in the specification of **Cummins** have no autoimmune basis (e.g., cancers, acne, warts). Instead, most of the specification and all of the claims describe the treatment of conditions of viral origin. In addition, the vast majority of the conditions in **Cummins** supported by actual clinical data are veterinarian diseases such as canine lupus erythematosus. These points have been addressed by Dr. Wolinsky, who states:

> The experimental database relied upon in the **Cummins** patent is extremely limited. Whereas reasonable information is provided for the treatment of shipping fever in cattle, **Cummins**' additional claims regarding the administration of interferon to treat viral and inflammatory disease are based on pure speculation or limited anecdotal evidence.

The specification of the instant application, in contrast, has targeted a much broader spectrum of autoimmune diseases including 27 cases of multiple sclerosis and 18 cases of treating autoimmune conditions in animals in which the type one interferon is orally administered and immediately ingested.

Cummins provides only one anecdotal example of the use of interferon treatment in multiple sclerosis. This one anecdotal example, however, is neither conclusive nor enabling. The case

involved a 30-year-old Caucasian female who received treatment for twenty-one days and had no recurrence of neurologic symptoms for nine months. As any clinician can attest, multiple sclerosis is a highly variable disease with unpredictable periods of remission and relapse. Therefore, a single individual having no recurrence of symptoms for nine months is hardly remarkable and certainly not conclusive. This is not an unusual period of remission in an untreated multiple sclerosis patient. In contrast, the instant application contains data from 27 multiple sclerosis patients. Furthermore, there are no claims in the **Cummins** patent regarding multiple sclerosis or any other autoimmune disease, most likely because the data failed to enable such claims.

In addition to the multiple sclerosis patient, **Cummins** also cites cases of treatment involving malignant lymphoma mesothelioma, and apthous stomatitis which are entirely anecdotal. One having ordinary skill in the art would find these anecdotal examples to be literally incredible and unclear in interpretation of therapeutic efficacy and therefore nonenabling. In fact, this section of **Cummins** reads more like a marketing tract than a description of a conclusive clinical investigation.

The Applicant's contention that **Cummins** fails to anticipate the instant invention is substantiated by both Rule 1.132 Declarations. On page 2 of his Declaration, Dr. Wolinsky states:

> It is my considered opinion that none of the supporting evidence in **Cummins** is in any way adequate to allow a reasonable physician, i.e. a person with ordinary skill in the art, with a reasonable expectation of being able to successfully utilize oral administration of interferon for the treatment of inflammatory autoimmune disease such as multiple sclerosis.

Likewise, Dr. Lindsey states on pages 4-5 in his Rule 1.132 Declaration,

> ... the extremely limited clinical anecdotes presented in **Cummins** would not provide a person with ordinary skill in this art with a reasonable expectation of being able to successfully treat an autoimmune disease such as multiple sclerosis by orally administering alpha interferon.

Cummins also fails to anticipate the instant invention because of significant differences in the routes of administration of the interferon. Applicant's claim 1 recites, "A method of treating an auto-immune disease in an animal comprising the step of orally administering a type one interferon such that the type one interferon is ingested immediately upon administration (emphasis added)". This differs considerably from the method of **Cummins** as stated in col. 4, lines 37-43:

> It is critical that the interferon be administered in a dosage form adapted to assure maximum contact of the interferon in said dosage form with the oral and pharyngeal mucosa of the human or animal. Contact of interferon can be enhanced by maximizing residence time of the treatment solution in the oral or pharyngeal cavity. Thus, best result seem to be achieved in human patients when the patient is requested to hold said solution of interferon in the mouth for a period of time.

With regards to this issue Dr. Lindsey stated the following in his Rule 1.132 Declaration:

> **Cummins** stressed that contact with the oral or pharyngeal mucosa should be maximized... Clearly, contact with the gastric or intestinal mucosa was regarded as inconsequential, while contact with the oral or pharyngeal mucosa was essential...

Thus, it is clear that the **Cummins, Jr.** patent actually argues against the immediate ingestion of the interferon, actually teaches away from the current application and certainly does not provide each and every component of the Applicant's claimed methods.

The Examiner has also cited column 13 of **Cummins** as anticipating the instant invention. Column 13 is concerned with the formation of the interferon in the form of lozenges, chewable vitamins, mouthwash, syrup, or effervescent tablet. All of these formulation are designed to maximize contact with the oral and pharyngeal mucosa rather than to facilitate ingestion. For example,

in the case of the lozenge, **Cummins** states: "The patient is instructed to hold the lozenge in his mouth until it is completely dissolved to release the interferon component for contact with the oral mucosa."

In the Applicant's animal experiments, animals were fed (implying ingestion) interferon using a 20 gauge ball point needle "placed in the posterior oropharynx (Page 29, line 23)." Such placement results in delivery of the interferon directly to the distal esophagus, stomach and small intestine and bypasses contact with the oral and pharyngeal mucosa. This was verified experimentally by injecting Evan's Blue during routine feeding and sacrifice which confirmed that no contact with the oral or pharyngeal mucosa occurred. Dr. Wolinsky states on page 3 of his Declaration, "Convincing data that shows that delivery of the dose of oral interferon must be into the duodenal small intestine in order to be effective is presented..."

In Applicant's clinical studies with human subjects, the interferon was ingested which allowed brief exposure to the oral mucosa. However, this mucosal contact was minimal unlike in **Cummins** where attempts were made to maximize the contact with the oral and pharyngeal mucosa. This distinction between

Cummins' method and the instant invention is further noted by Dr. Lindsey on page 3 of his declaration:

> In Applicant's clinical studies with human subjects, the interferon was "ingested," which briefly exposed the oral mucosa to the interferon, but no attempts at maximizing contact with the oral mucosa were made nor would there have been any significant absorption of the alpha-interferon through the oral or pharyngeal mucosa.

In reference to the Applicant's method of administration, Dr. Wolinsky states on page 3 of his Declaration, "...this specific route of administration is quite unlike the oral mucosa swish technique described by **Cummins**." In addition, in **Cummins**, the interferon was sometimes discharged from the patient's mouth without ingestion (Col. 12, lines 27-29 of **Cummins**). In contrast, the Applicant's specification shows the necessity of interferon interacting with intestinal sites and Peyer's patches.

Finally, the dosages of interferon used by **Cummins** were smaller than in the instant application. In **Cummins**, the dosages of interferon are given in Col. 4, lines 24-32. These range from 0.01 to 5 I.U./lb. per day. The instant application, in contrast, uses dosages ranging from 5 I.U./kg to about 50,000 I.U./kg (which corresponds to 2.3 I.U./lb. to 23,000 I.U./lb.,), a much greater range than that of **Cummins**. More importantly, as pointed out by Dr. Lindsey, "...the doses found to be most effective are around two orders of

magnitude, or 100 times, higher that the maximum dose recommended by **Cummins**." Another point of departure from **Cummins** is discussed by Dr. Wolinsky on page 4 of his Rule 1.132 Declaration:

> Particularly important is Applicant's demonstration of an unusual dose-response relationship for oral administration of type one interferons to have any effect. It is meticulously demonstrated that both doses that are too low and doses that are too high lack any clinical benefit in animal models of human autoimmune disease... Applicant's work thus defines a likely range of doses of type one oral interferons that would be clinically efficacious in humans with multiple sclerosis and other inflammatory autoimmune diseases.

No such relationship is discussed in **Cummins**.

In reference to the described dosage regimen, the Examiner cites col. 5, lines 50-55 of **Cummins** which states:

> Daily dosage of interferon can be administered as a single dose or, preferably, it is divided and administered in a multiple-dose daily regimen. A staggered regimen, for example one to three days of treatment per week or month, can be used as an alternative to continuous daily treatment." (Emphasis added).

The Examiner contends that this anticipates the every other day administration of interferon described in the instant application. The Applicant respectfully disagrees. The first portion teaches a multiple-dose daily regimen rather than an alternate day regimen.

In the second section, the spacing of the one to three days of treatment per week or month is not discussed and the **Cummins** patent is therefore non-enabling in that regard.

Thus, the Applicant maintains that such substantial differences exist between the method of **Cummins** and the claims presented in the instant application that the **Cummins** patent fails to anticipate the current claims. Therefore, based on the arguments herein and in the two Declarations under 37 CFR 1.132, the applicant respectfully requests that the rejection of claims 1-4, 6-11, and 13-20 under 35 U.S.C. §102(b) as anticipated by **Cummins** be withdrawn.

B. 35 U.S.C. §103

(1) Claims 5 and 12 stand rejected under 35 U.S.C. §103 as obvious over **Cummins, Jr.** (U.S. Patent 5019382). Specifically, in the Office Action mailed July 16, 1997, the Examiner cited Col. 5, lines 50-55 as suggesting an alternating day dosage regimen. The Applicant respectfully disagrees.

The appropriate section of **Cummins** has be quoted *supra*. The section refers to a multiple-dose daily regimen rather than an alternate day regimen. While a regimen of one to three

days per week or month is discussed, this is alluded to as a less preferred mode of administration by **Cummins**. In addition, the specific spacing of the treatment is not discussed. It is unclear whether this section refers to three continuous days on treatment followed by a period of day without treatment or whether it refers to single days of treatment separated by days without treatment. As such, the section is non-enabling and does not render obvious claims 5 and 12. Undue experimentation would be necessary to try the other possible combinations days on therapy versus days off therapy. Therefore, the Applicant respectfully requests that the rejection of claims 5 and 12 under 35 U.S.C. §103 as obvious over **Cummins** be withdrawn.

(2) Claims 1-20 stand rejected under 35 U.S.C. §103 as obvious over by **Cummins, Jr.** (U.S. Patent 5019382) in view of **Shibutani** et al. (Iyakuhin Kenkyu, vol . 18(4), pp. 571-82, 1987). Based on the interferon toxicity data of **Shibutani,** the Examiner argues that interferon would be tolerated well at high doses, and therefore, it would be obvious to combine the **Shibutani** with **Cummins** to devise the higher dose regimen described in the instant application. The Applicant respectfully disagrees.

The **Shibutani** abstract describes the lack of toxicity of human beta interferon in mice and rats. It by no means teaches or suggests a method of oral administration of a type one interferon in the treatment of autoimmune diseases. In addition, the lack of toxicity of higher doses does not imply that those doses would be efficacious in the treatment of autoimmune diseases, and further undue experimentation would be necessary to determine if higher doses would indeed be useful in the treatment of a particular disease.

In the Rule 1.132 declarations, the issue of dosage is described in some detail. The instant application is precise and meticulous is its delineation of a dose-response relationship for oral administration and ingestion of type one interferons. This relationship is somewhat exceptional. No overlap exists in the dosages used by **Cummins** and the doses found to most effective in the present invention which are about two order of magnitude greater than those used by **Cummins**. Furthermore, as previously mentioned, Dr. Wolinsky pointed out that the Applicant has "meticulously demonstrated that both doses that are too low and doses that are too high lack any clinical benefit." One skilled in art would not be able to determine this from the combination of

Cummins and **Shibutani** without extensive, undue experimentation.

In addition, as described *supra*, the higher dose regimen is only one of several differences between **Cummins** and the present application. The differences in route of administration, dosage regimen, and degree of clinical enablement have already been discussed in depth previously herein. The human clinical data in the **Cummins** specification is highly anecdotal and non-enabling. A person having ordinary skill in the art would not be likely to believe **Cummins**' rhetoric regarding the importance of contact with the oropharyngeal membranes nor would such a person be motivated to make the instant invention based on this work. These deficiencies are not resolved by the addition of the **Shibutani** abstract.

In reference to the combination of **Cummins** and **Shibutani**, Dr. Lindsey writes:

> Clearly, one with ordinary skill in the art of autoimmune pathophysiology and treatment would not expect clinical efficacy in humans from the oral administration of alpha interferon after having read the **Cummins** and/or **Shibutani** et al. references. In fact, the opposite expectation that interferon would have no effect is more reasonable. Interferon is a protein and proteins are broken down in the gastrointestinal tract. Thus, a person having ordinary skill in this art would expect interferon to be inactive when swallowed. Hence, the claimed methods not only are not obvious to one of ordinary skill, they are also counterintuitive.

On this same point, Dr. Wolinsky writes, "...any person with ordinary skill in this art would expect interferons to be biologically inert after being swallowed. Thus, based on the arguments herein and the two 37 CFR 1.132 Declarations, the combination of **Cummins** and **Shibutani** fail to make the present invention obvious, the applicant respectfully requests that the rejection of claims 1-20 under 35 U.S.C. §103 as obvious over **Cummins** in view of **Shibutani** be withdrawn.

C. 35 U.S.C. §112

(1) In the Response to office Action filed October 7, 1997, Claims 1, 8, 19 were amended to change "ingestion after oral administration" to "ingestion immediately upon oral administration." This was done to indicated more clearly that the intent of oral administration in the instant application was for ingestion of the interferon rather than retention in the mouth for a period of time as in **Cummins**. In the Office Action mailed December 17, 1997, the Examiner declared that the words "immediately upon" in reference to ingestion after oral administration were not enabled by the specification and rejected claims 1-12 and 19-20 under 35 U.S.C. §112. The Applicant respectfully disagrees.

The phrase "ingestion 'immediately upon oral administration" is clearly supported in Applicant's specification by the description of the animal experiments in which interferon was administered directly to the distal esophagus, stomach, and small intestine via a 20 gauge ball point needle. Applicant's specification, therefore, contains data teaching a person having ordinary skill in this art how to use the claimed methods. Accordingly, the applicant respectfully requests that the 35 U.S.C. §112 rejection of claims 1-12 and 19-20 be withdrawn.

Respectfully submitted,

Date: March 1, 1999

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APPENDIX A:

CLAIMS ON APPEAL

1. A method of treating an auto-immune disease in an animal comprising the step of orally administering a type one interferon to said animal such that the type one interferon is ingested immediately upon oral administration.

2. The method of claim 1, wherein said interferon is selected from alpha-interferon and beta-interferon.

3. The method of claim 2, wherein said interferon is selected from the group consisting of human recombinant interferon, rat interferon and murine interferon.

4. The method of claim 2, wherein said interferon is administered in a dosage of from about 50 I.U./kg to about 25,000 I.U./kg.

5. The method of claim 1, wherein said interferon is administered every other day.

6. The method of claim 1, wherein said animal is a human.

7 The method of claim 1, wherein said auto-immune disease is selected from the group consisting of multiple sclerosis, rheumatoid arthritis, diabetes mellitus, psoriasis, organ-specific auto-immune diseases, chronic inflammatory demyelinating polyradiculoneuropathy and Guillain-Barré syndrome.

8. A method of decreasing the severity or frequency of a relapse of multiple sclerosis in a human comprising the step of orally administering a type one interferon to said animal such that the type one interferon is ingested immediately upon oral administration.

9. The method of claim 8, wherein said interferon is selected from alpha-interferon and beta-interferon.

10. The method of claim 8, wherein said interferon is selected from the group consisting of human recombinant interferon, rat interferon and murine interferon.

11. The method of claim 8, wherein said interferon is administered in a dosage of from about 5 I.U./kg to about 50,000 I.U./kg.

12. The method of claim 8, wherein said interferon is administered every other day.

13. A method of reducing inflammation associated with an auto-immune disease in an animal comprising the step of orally administering a type one interferon to said animal such that the type one interferon is ingested after oral administration.

14. The method of claim 13, wherein said interferon is selected from alpha-interferon and beta-interferon.

15. The method of claim 13, wherein said interferon is selected from the group consisting of human recombinant interferon, rat interferon and murine interferon.

16. The method of claim 15, wherein said interferon is administered in a dosage of from about 50 I.U./kg to about 25,000 I.U./kg.

17. The method of claim 13, wherein said animal is a human.

18. The method of claim 13, wherein said auto-immune disease is selected from the group consisting of multiple sclerosis, rheumatoid arthritis, diabetes mellitus, psoriasis, organ-specific auto-immune diseases, chronic inflammatory demyelinating polyradiculoneuropathy and Guillain-Barré syndrome.

19. A method of decreasing the levels of a cytokine in an individual having multiple sclerosis, comprising the step of orally administering a type one interferon to said individual, wherein said cytokine is selected from the group consisting of TGF-β, IL-2, IL-10, IFN-γ and ICAM-1; and wherein said type one interferon is ingested immediately upon oral administration.

20. The method of claim 19, wherein said interferon is administered in a dosage of from about 166 I.U./kg to about 500 I.U./kg.

United States Patent [19]

Cummins, Jr.

[11] **Patent Number: 5,019,382**

[45] **Date of Patent: May 28, 1991**

[54] **TREATMENT OF IMMUNO-RESISTANT DISEASE WITH LOW-DOSE INTERFERON**

[75] Inventor: **Joseph M. Cummins, Jr.**, Amarillo, Tex.

[73] Assignee: **The Texas A&M University System**, College Station, Tex.

[21] Appl. No.: **465,527**

[22] Filed: **Jan. 17, 1990**

Related U.S. Application Data

[63] Continuation of Ser. No. 927,834, Nov. 6, 1986, abandoned.

[51] **Int. Cl.**[5] **A61K 37/66**

[52] **U.S. Cl.** 424/85.4; 424/85.6; 424/85.7

[58] **Field of Search** 424/85.4, 85.6, 85.7

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(List continued on next page.)

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[57] **ABSTRACT**

Neoplastic disease, hyperallergenicity, autoimmune disorders characterized by chronic tissue degenerative inflammation and immuno-resistant viral infections are treated by the administration of interferon at a dosage of about 0.1 to about 5 IU/lb per day by contacting said interferon with oral/pharyngeal mucosa. Interferon is administered in solution or in a novel solid unitary dosage form adapted to be dissolved in saliva when placed in the mouth.

14 Claims, No Drawings

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TREATMENT OF IMMUNO-RESISTANT DISEASE WITH LOW-DOSE INTERFERON

BACKGROUND OF THE INVENTION

This invention relates generally to an improved method of treating diseases of immuno-pathologic etiology in warm-blooded vertebrates using interferon in low oral dosages. This invention also relates to the use of interferon in low oral dosages to potentiate disease-corrective immune responses in warm-blooded vertebrates afflicted with immuno-resistant diseases characterized by apparent hyperactive or hypoactive immune system function.

"Interferon" is a term generically comprehending a group of vertebrate glycoproteins and proteins which are known to have various biological activities, such as antiviral, antiproliferative, and immunomodulatory activity at least in the species of animal from which such substances are derived. The following definition for interferon has been accepted by an international committee assembled to devise a system for the orderly nomenclature of interferons: "To qualify as an interferon a factor must be a protein which exerts virus nonspecific, antiviral activity at least in homologous cells through cellular metabolic processes involving synthesis of both RNA and protein." *Journal of Interferon Research*, 1, pp. vi (1980). "Interferon" as used herein in describing the present invention shall be deemed to have that definition.

Since the first descriptions of interferon by Isaacs and Lindeman [See, *Proc. Roy Soc. London (Ser. B)*, Vol. 147, pp. 258 *et seq.* (1957) and U.S. Pat. No. 3,699,222], interferon has been the subject of intensive research on a worldwide basis. The literature is replete with publications concerning the synthesis of interferon, its proposed molecular characterizations, its clinical applications and proposed mechanisms of its antitumor, antiviral, and immune system activities.

Because of the intensity and disparate origins of research concerning interferon and its characteristics and uses, there exists a substantial lack of uniformity in such matters as classification of interferon types. There are also numerous, sometimes contradictory, theories concerning the mode of action of interferon in producing clinical effects.

Although originally isolated from cells of avian origin (chick allantoic cells), interferon production has been observed in cells of all classes of vertebrates, including mammals, amphibians, birds and reptiles. Interferon production by vertebrate cells is seldom spontaneous but is often readily "induced" by treatment of cells (in vivo or in vitro) with a variety of substances including viruses, nucleic acids (including those of viral origin as well as synthetic polynucleotides), lipopolysaccharides, and various antigens and mitogens.

Interferons have generally been named in terms of the species of animal cells producing the substance (e.g., human, murine, or bovine), the type of cell involved (e.g., leukocyte, lymphoblastoid, fibroblast) and, occasionally, the type of inducing material responsible for interferon production (e.g., virus, immune). Interferon has been loosely classified by some researchers according to induction mode as either Type I or Type II, with the former classification comprehending viral and nucleic acid induced interferon and the latter class including the material produced as a lymphokine through induction by antigens and mitogens. More recently, the international committee devising an orderly nomenclature system for interferon has classified interferon into types on the basis of antigenic specificities. In this newer classification, the designations alpha (α), beta (β), and gamma (γ) have been used to correspond to previous designations of leukocyte, fibroblast, and type II (immune) interferons, respectively. Alpha and beta interferons are usually acid-stable and correspond to what have been called type I interferons; gamma interferons are usually acid-stable and correspond to what has been called type II interferons. The international committee's nomenclature recommendations apply only to human and murine interferons. *Journal of Interferon Research*, 1 pp. vi (1980).

In its earliest applications, interferon was employed exclusively as an antiviral agent and the most successful clinical therapeutic applications to date have been in the treatment of viral or virus-related disease states. It became apparent, however, that exogenous interferon was sometimes capable of effecting regression or remission of various metastatic diseases. An overview of current clinical trials of interferon as an antiviral and antiproliferative therapeutic agent is contained in *Interferon: In Vivo and Clinical Studies*, Volume 4, Eds: N. B. Finter and R. K. Oldham, Academic Press, New York, 1985.

The clinical agent of choice for the present is human leukocyte interferon, "mass-produced" by procedures involving collection and purification of vast quantities of human buffy coat leukocytes, induction with virus, and isolation from culture media.

In the work described above, interferon has been administered parenterally, i.e., intramuscularly and intradermally, with some successful topical and intranasal usages having been reported. It has seldom been administered intravenously because of substantial adverse effects attributable to "contaminants" in crude and even highly purified isolates.

As discussed above, there has been a significant research effort directed to the evaluation of therapeutic effects of interferon for a wide variety of diseases having an auto-immuno-pathologic basis. Before applicant's first report of successful oral administration of interferon in his U.S. Pat. application Ser. No. 415,525 (now U.S. Pat. No. 4,462,985), there was no recognition in the art of the potential offered by oral administration of interferon. The generally held belief was that interferon could not survive the digestive conditions of the upper alimentary canal.

Since applicant's first disclosure of the immunotherapeutic benefit achievable via oral administration of interferon of heterologous mammalian species, he has continued to investigate the efficacy of orally administered interferon. In U.S. Pat. No. 4,497,795, issued Feb 5, 1985, applicant described and claimed the use of interferon administered orally or via intravenous administration to stimulate appetite and feed efficiency of bovine and porcine species. More recently applicant has described in now pending U.S. applications the use of interferon at dosages less than about 5 IU per pound of body weight for increasing feed efficiency and food utilization in warm-blooded vertebrates, for preventing and treating shipping fever, and for enhancing vaccine efficiency.

Human alpha-interferon has been marketed under the trademark Agriferon ® by Immunomodulator Laboratories, Inc. ("IML") of Stafford, TX for veterinary use in Texas since February 1985. The product is sold for

oral administration to cattle to promote growth and feed efficiency and to prevent or treat viral respiratory infections. IML began selling an alpha-interferon product for horses in 1986. Both products are sold under a license of my U.S. Pat. 4,462,985.

SUMMARY OF THE INVENTION

Interferon contacting the oral and/or pharyngeal mucosa, in amounts of less than 5 IU/lb of body weight per day is consistently effective to potentiate disease-corrective immune responses in vertebrates afflicted with immuno-resistant disease states characterized by apparent hyperactive or hypoactive immune system function. Treatment in accordance with the present invention has been shown to effect remission of neoplastic disease, hyperallergenicity, immuno-resistant or immuno-debilitating viral infections and autoimmune disorders characterized by chronic tissue degenerative inflammation.

DETAILED DESCRIPTION OF THE INVENTION

The clinical agent of choice for use in the present invention is human leukocyte interferon (human alpha-interferon), "mass-produced" by procedures involving collection and purification of quantities of human buffy coat leukocytes, induction of interferon production with virus, and isolation of culture media. (See "Preparation of Human Alpha-Interferon" below.) Also acceptable for use in accordance with present intention are human alpha-interferon products produced by recombinant DNA technology and now commercially available from Schering-Plough (as Intron®) and Hoffmann-LaRoche (as Roferon®) and approved by the FDA for treatment (parenterally) of hairy cell leukemia of man. Such recombinant interferon products are believed to be particularly effective when used in combination. Gamma interferon is also available by recombinant technology and is presently undergoing clinical trials by Genentech and others. Fibroblast interferon (beta-interferon) can be prepared in accordance with Example 1 in applicant's U.S. Pat. No. 4,462,985 issued July 31, 1984, the disclosure of which is hereby expressly incorporated by reference.

Interferon of human and murine origins has been quantified in the art in terms of International Units ("IU"). As used herein, a "unit" of interferon (to be distinguished from "IU") shall mean the reciprocal of a dilution of interferon-containing material that, as determined by assay, inhibits one-half the number of plaques of a challenge virus, the challenge virus being the vesicular stomatitis virus ("VSV"). So quantified a "unit" of interferon is routinely found to be about one-tenth the quantity of interferon represented by one "IU." In other words, for the purpose of defining the present invention, 1 unit $\simeq$0.1 IU.

The present invention relates to an improved method of treatment of immuno-resistant disease states with interferon. The present invention is directed to the treatment of diseases in warm-blooded vertebrates, particularly certain diseases which the immune system of many species is poorly equipped to handle, as evidenced by either a lack of disease defeating response and/or an apparently misdirected immune response resulting in a chronic tissue degenerative inflammatory condition or other physical complications. While there has been a significant research effort directed to the use of interferon for treatment of such diseases, reported results, although positive overall, have been inconsistent. The principle reason for such inconsistency in view of my most recent research efforts is that earlier investigators have failed to define optimum dosage and route of interferon administration.

The present invention is based on applicant's discovery that interferon can be used as a consistently effective therapeutic agent for treatment of diseases having an immunopathologic basis—characterized by inadequate immune response and persistence of the disease or by an apparent hyperactive immune response resulting in tissue degenerative inflammatory conditions and related physical manifestations. Applicant has found that interferon, contacting the oral and pharyngeal mucosa in amounts from about 0.01 to about 5 IU/lb of body weight per day, is consistently efficacious for the treatment of diseases to which the immune system of many warm-blooded vertebrates does not effectively respond.

Disease conditions treated in accordance with the present invention include apparent autoimmune disorders characterized by a chronic tissue degenerative inflammatory condition. Diseases so characterized include multiple sclerosis, rheumatoid arthritis, stomatitis, and lupus erythematosus. Treatment of such disease is in accordance with the present invention comprises administering interferon at a dosage of 0.01 to about 5 IU/lb per day in a dosage form adapted to promote contact of said dosage of interferon with the oral and pharyngeal mucosa of said animal. Preferably, the dosage of interferon is from 0.1 to about 4.0 IU/lb per day, more preferably 0.5 to about 1.5 IU/lb of body weight per day. Alpha interferon, derived from tissue culture or by recombinant DNA techniques, is a preferred therapeutic agent in accordance with this invention. Alpha interferon can be administered alone or in combination with beta interferon or gamma interferon.

It is critical that the interferon be administered in a dosage form adapted to assure maximum contact of the interferon in said dosage form with the oral and pharyngeal mucosa of the human or animal undergoing treatment. Contact of interferon with the mucosa can be enhanced by maximizing residence time of the treatment solution in the oral or pharyngeal cavity. Thus, best results seem to be achieved in human patients when the patient is requested to hold said solution of interferon in the mouth for a period of time. Contact of interferon with the oral and pharyngeal mucosa and thereafter with the lymphatic system of the treated human or animal is unquestionably the most efficient method administering immunotherapeutic amounts of interferon.

Another disease condition responding to treatment in accordance with the present invention is neoplastic disease. Thus, the administration of interferon in accordance with the above description can, alone or in combination with other drugs or therapy, help effect remission of cancers such as malignant lymphoma, melanoma, mesothelioma, Burkitt lymphoma and nasopharyngeal carcinoma and other neoplastic diseases, especially those of known or suspected viral etiology. Based on the results observed to date, it is believed that applicant's presently described method of treatment will similarly help effect remission of Hodgkin's Disease and leukemia.

Other disease conditions responding to treatment in accordance with the present invention are infectious diseases of viral origin in, for example, human, avian, porcine, canine and feline species. Significantly, viral

infection typically exhibiting persistent resistance to treatment have shown a dramatic response to treatment with interferon in low doses contacting the oral and pharyngeal mucosa of infected patients. Beneficial results have been attained utilizing the present method to treat dogs having canine parvovirus and canine herpesvirus infections. Further, feline leukemia and feline infectious peritonitis have been shown to be particularly susceptible to treatment with alpha interferon and beta interferon in accordance with this invention.

Exemplary of human viral infections showing remarkable response to treatment in accordance with the present invention are infections of human rhinovirus (common cold), herpes simplex I virus (cold sores) and human papovavirus (warts). Based on treatment results to date, it is expected that contact of interferon at low dosage with the oral and pharyngeal mucosa will provide an effective treatment for Acquired Immune Deficiency Syndrome (AIDS) and disease conditions having the herpes simplex II virus as the causative agent. A patient experiencing a condition of viral myocarditis has responded favorably to the present treatment. Warts often dissipate within six to eight weeks after initiating treatment in accordance with this invention.

Other afflictions responding to contact of low dosage interferon are hyperallergenic conditions such as asthma. One "side effect" noted by patients treated in accordance with this invention is improved skin complexion. Thus, administration of interferon in dosages of about 0.01 to about 5 IU/lb of body weight per day is effective to treat acne, specifically and improve human skin complexion generally.

Further, stimulating the immune system by oral contact with low dosage interferon is believed to assist the body in fighting bacterial infection. Treatment in accordance with this invention alone or in combination with therapeutic amounts of antibiotics can be especially effective in knocking down infections of antibiotic resistant microorganisms.

Administration of interferon in accordance with the present invention is preferably continued until the symptoms of the disease condition being treated subside. This can range from a period of one day, for example, where a human rhinovirus is the disease causative agent, to a period of up to six months for treatment of neoplastic disease. Rheumatoid arthritis patients are pain free within 2 to 10 days of initiating treatment in accordance with the present invention. However, treatment of that disease is preferably conducted by administration of interferon for up to about three (3) months.

Daily dosage of interferon can be administered as a single dosage or, preferably, it is divided and administered in a multiple-dose daily regimen. A staggered regimen, for example one to three days treatment per week or month, can be used as an alternative to continuous daily treatment.

Interferon can be administered in accordance with this invention in either a liquid (solution) or solid dosage form. Thus interferon can be administered dissolved in a buffered aqueous solution typically containing a stabilizing amount (1-5% by weight) of blood serums. Exemplary of a buffered solution suitable as a carrier of interferon administered in accordance with this invention is phosphate buffered saline prepared as follows:

A concentrated (20x) solution of phosphate buffered saline (PBS) was prepared by dissolving the following reagents in sufficient water to make 1,000 ml of solution: sodium chloride, 160 grams; potassium chloride, 4.0 grams; sodium hydrogen phosphate, 23 grams; potassium dihydrogen phosphate, 4.0 grams; and optionally phenol red powder, 0.4 grams. The solution is sterilized by autoclaving at 15 pounds pressure for 15 minutes and then diluted with additional water to a single strength concentration prior to use.

Alternatively the interferon can be formulated into flavored or unflavored solutions or syrups using a buffered aqueous solution of interferon as a base with added caloric or non-caloric sweeteners, flavor oils and pharmaceutically acceptable surfactant/dispersants.

It is also contemplated by the present invention to provide interferon in a solid dosage form such as a lozenges adapted to be dissolved upon contact with saliva in the mouth with or without the assistance of chewing. Such a unitary dosage form is formulated to release about 1 to about 1500 IU of interferon upon dissolution in the mouth for contact with the oral and pharyngeal mucosa. Thus a unitary dosage form of interferon in accordance with this invention can be prepared by art-recognized techniques for forming compressed tablets such as chewable vitamins. Similarly, interferon can be incorporated into starch-based gel formulations to form a lozenge which will dissolve and release interferon for contact with the oral mucosa when held in the mouth. Solid unitary dosage forms of interferon for use in accordance with the present invention can be prepared utilizing art recognized dosage formulation techniques. The pH of such formulations can range from about 4 to about 8.5. Of course, in processing to such unitary dosage forms one should avoid heating a pre-dosage form formulation, after addition of interferon, above about 50° Centigrade.

Preparation of Human Aloha-Interferon

Human alpha-interferon can be prepared through the following procedure, commonly referred to as the Cantell procedure. The process begins with packs of human leukocytes, obtained in this case from the Gulf Coast Regional Blood Center, Houston, Texas. The buffy coats in these packs are pooled into centrifuge bottles, and then are diluted with 0.83% ammonium chloride. The mixture is incubated for 15 minutes with intermittent shaking, and is then centrifuged for 20 minutes at 2000 rpm. The supernatant is discarded, and the cell pellets are resuspended with a minimal volume of sterile phosphate buffered saline (PBS). The mixture is then diluted with ammonium chloride and centrifuged. The supernatant is again discarded, and the remaining cell pellets are resuspended with a minimal volume of a tissue culture medium such as Minimal Essential medium (MEM), available from KC Biological. The cell concentration is determined with a Coulter counter.

Interferon induction takes place in glass or plastic bottles. The induction medium contains MEM 75 mM Hepes (available from Calbiochem), 75 mM Tricine (available from Sigma Chemical Co.), human agamma serum (18 mg/ml), and gentamycin sulfate (from M.A. Bioproducts; 50 mcg/ml). The cells are added to the induction vessels at a final concentration of about 5 to 10 million cells per milliliter. The induction vessel is incubated in a 37° C. water bath, and interferon alpha is added as a primer.

After two hours, Sendai virus is added to the induction mixture. This causes alpha interferon to be produced in the supernatant by the leukocytes. After a 12-18 hour incubation time, the induction mixture is

centrifuged. The cells are discarded, and the supernatant is then purified.

The crude interferon is chilled to 10° C. or below in an ice bath. Five molar potassium thiocyanate is added to obtain a final concentration of 0.5M. This solution is stirred for 15 minutes, and then its pH is lowered to 3.3 by adding hydrochloric acid. The mixture is then centrifuged at 2800 rpm for 30 minutes, and the supernatant is discarded.

The pellets are then resuspended in 95% ethanol and are stirred for 15 minutes. This suspension is centrifuged at 2800 rpm for 20 minutes, and the pellets are discarded. The pH of the supernatant is then adjusted to 5.8 with sodium hydroxide. The mixture is stirred for 10 minutes, and then centrifuged at 2800 rpm for 20 minutes. The pellets are discarded. The pH of the supernatant is then adjusted to 8 with sodium hydroxide. This solution is stirred for 10 minutes, followed by centrifugation at 2800 rpm for 20 minutes. The supernatant is discarded, and the pellets are resuspended with 0.5M potassium thiocyanate in a 0.1M sodium phosphate buffer. This suspension is stirred at 4° C.

Next, the suspension is centrifuged at 2800 rpm for 20 minutes, and the pellets are discarded. The pH of the supernatant is adjusted to 5.3 with hydrochloric acid. After stirring for 10 minutes and centrifugation, the pH of the supernatant is adjusted to 2.8 with hydrochloric acid, followed by further stirring for 20 minutes. This mixture is centrifuged at 2800 rpm, and the resulting pellet is purified human alpha-interferon.

The pellet is resuspended with 0.5M potassium thiocyanate in 0.1M sodium phosphate buffer, having a pH of 8.0. It is then dialyzed against PBS at 4° C., with two changes of PBS. This mixture is then centrifuged and the precipitate is discarded. The remaining purified alpha interferon is sterilized by filtration through a 0.2 micron filter. A human alpha-interferon is produced in accordance with this procedure by Immuno Modulators Laboratories, Inc., Stafford, TX, and sold under the trademark Agriferon ® for use in cattle and Equiferon ® for use in horses.

Other procedures known to those skilled in the art are available for making interferons, such as human alpha-interferon and human gamma-interferon. For example, U.S. Pat. Nos. 4,376,821 and 4,460,685 disclose methods of making human gamma-interferon. A method of making bovine fibroblast (beta) interferon is disclosed in applicant's U.S. Pat. No. 4,462,985.

Clinical Studies

Tables 1–4 below summarize the results of clinical studies of the administration of interferon by veterinarians orally to 137 dogs and cats as of Nov., 1985. The studies were conducted with both human alpha-interferon and bovine beta-interferon. Tables 1–4 compare survival rates of pets with feline leukemia virus-associated diseases or canine parvovirus disease. Unequal numbers of pets were treated with each type of interferon; bovine beta-interferon was given to 78 pets and human alpha-interferon was given to 59 pets.

Bovine beta-interferon was produced in flasks of confluent monolayers of bovine fetal kidney (BFK) cells. Culture supernatant was harvested 24 hours after bluetongue virus induction of BFK cells. The supernatant was dialyzed 24 hours in a pH 2.0 buffer and for another 24 hours in a PBS (pH 7.4) buffer before interferon assay. Procedures for the assay and characterization of bovine beta-interferon were essentially as described by Rosenquist and Loan, *American Journal of Veterinary Research*, 28; 619–628, 1967. Interferon titers as "units" were expressed as the reciprocals of the dilutions that provided a 50% reduction in the number of VSV plaques as compared with the number in control cultures. The BFK cell culture interferon produced by this method had an average titer of 7,000 units per milliliter. Dogs were given bovine beta-interferon, 5–10 ml/dose, as least three times/day after a diagnosis of CPV disease. Cats positive by ELISA for feline leukemia virus and exhibiting clinical signs of disease were given 1 ml/10 lb of body weight 2–3 times daily for five days. After a five-day interval, cats were retreated at least once for another five days.

Human alpha interferon was obtained from IML, Inc. of Houston, TX. Cases were treated with lot AO26 applied at 6×10^6 IU/ml. Lot AO26 of human alpha interferon was diluted 1:150 in Eagles' minimum essential medium (MEM) and used as the stock solution from which 1 ml was further diluted 1:1000 with 1 liter of MEM for treatment. The usual dose of human alpha interferon was 4 IU/lb body weight given at least three times daily after a diagnosis of CPV disease was made. For feline leukemia, cats were treated with human alpha-interferon 2–3 times daily for five days as reported for bovine beta-interferon.

Significantly ($P<0.05$) more cats lived six and twelve months after diagnosis and treatment for feline leukemia virus if alpha-interferon was given, compared to treatment with bovine beta-interferon. Significantly ($P<0.05$) more dogs survived CPV disease when given alpha interferon (92%) compared to those dogs given bovine beta-interferon (69%).

TABLE 1

Summary of Survival Date from clinically ill cats positive for FeLV.

	Months After Treatment		
Treatment	1	6	12
Human alpha-IFN	25/33	21/32*	19/31*
Bovine IFN	26/36	15/36	13/36

Numerator = no. alive; denominator = no. treated.
*Cats given human alpha-IFN had significantly ($P < .05$) higher survival rates at 6 and 12 months after treatment than cats given bovine IFN. Significance was determined by Chi Square test.

TABLE 2

Percent survival of clinically ill cats positive for FeLV.

	Months After Treatment		
Treatment	1	6	12
Human alpha-IFN	76%	66%*	61%*
Bovine IFN	72%	42%	36%
Historical Control	<50%	<30%	—

Numerator = no. alive; denominator = no. treated.
*Cats given human alpha-IFN had significantly ($P < .05$) higher survival rates at 6 and 12 months after treatment than cats given bovine IFN. Significance was determined by Chi Square test.

TABLE 3

Response of CPV disease to treatment with bovine interferon or human alpha-interferon, by veterinarian.

Attending	Bovine IFN Beta		Human Alpha [illegible]	
Veterinarian	Lived	Died	Lived	Died
S	16/21	5/21	14/16	2/16
M	6/11	5/11	7/7	0/7
R	7/10	3/10	3/3	0/3

TABLE 3-continued

Response of CPV disease to treatment with bovine interferon or human alpha-interferon, by veterinarian.

Attending Veterinarian	Bovine IFN Beta		Human Alpha IFN	
	Lived	Died	Lived	Died
	29/42(69%)	13/42(31%)	24/26(92%)	2/26(8%)

Dogs treated with human alpha-interferon had a significantly ($P < .05$) higher survival rate compared to dogs treated with bovine IFN. Significance between groups was determined by Chi Square test.

TABLE 4

Treatment days for CPV disease.

Treatment	No. of Days	Average No. Treatment Days*	SD**	Survival Rate
Bovine IFN	42	3.31	1.95	69%
Human alpha IFN	26	2.75	0.92	92%

*Calculated on surviving dogs only.
**Standard deviation of the mean treatment days.

Canine Heroesvirus Challenge of Newborn Dogs

Canine herpesvirus infection of dogs less than one week of age are invariably fatal, but older pups usually survive. Interferon has been successfully used to treat viral infections of many species. These studies were conducted to assess the efficacy of interferon in canine herpesvirus (CHV) inoculated puppies.

Five (5) pregnant bitches were obtained from a USDA licensed supplier and were housed in a USDA approved research facility in Canyon, Texas. After the pups whelped, they were inoculated with 6.3 log 10 units of virulent CHV obtained from Dr. Richard Mock of the Texas A&M University Veterinary Medical Diagnostic Laboratory (TVMDL) in Amarillo, Texas. Human alpha-interferon (HAI) or placebo was given to pups orally as treatment in an effort to increase the survival rate of the CHV inoculated pups. Each litter was divided into control and treated animals. The procedures and schedule for each litter are discussed below. All dead animals were necropsied at TVMDL, Amarillo, TX.

LITTER 1

Nine (9) pups were inoculated orally with CHV on the day of birth. Interferon was given at 6–10 units (1X), or ten times the dosage at 60–100 units (10X). Three pups were given 0.5 ml placebo, 3 pups were given 0.5 ml HAI (1X), and 3 pups were given 0.5 ml of a 10X concentrate of HAI orally twice daily for 7 days (if they lived that long). The 3 controls died 5, 6, and 8 days after CHV inoculation. The 3 pups given HAI (1X) lived 7, 7, and 9 days and the 3 pups given 10X HAI lived 6, 7, and 7 days after CHV inoculation.

Pups given HAI (1X) lived an average of 1.3 days longer than controls, but the longer survival time was not statistically significant. The higher dosage, HAI (10X), did not provide a survival benefit over the lower dosage, but instead pups given the higher dosage died, on the average, one day sooner.

LITTER 2

Eight (8) pups were inoculated with CHV orally 2 days after birth. Interferon was given at 6–10 units (1X), or ten times the dosage (10X), or 1/10th the dosage (1/10X). All interferon was given orally after dilution in PBS. Two (2) pups were given 0.5 ml PBS, 2 were given 0.5 ml HAI at 1/10th concentration, 2 were given HAI (1X), and 2 were given a 10X concentrate of HAI. All treatments were given orally twice daily for 5 days starting 1 day before CHV inoculation. The 2 controls died <1 and 9 days after CHV inoculation and the HAI treated (1/10th dose, full dose, 10X dose) pups died 8 and 9, 5 and 9, 8 and 8 days after CHV inoculation, respectively.

No benefit from treatment at any dosage was seen. The death of a control pup within a day after CHV inoculation was probably not related to CHV inoculation.

LITTER 3

Nine (9) pups were inoculated with CHV orally 3 days after birth. Two pups were given 0.5 ml PBS, 2 were given 0.5 ml HAI (1X), 2 were given 1/10th dose HAI, and 3 were given 2 IU of recombinant human alpha-interferon from Schering-Plough. All treatments were given orally twice daily for 5 days starting two days before CHV inoculation. Both pups given HAI (1X) survived until necropsied 19 days after CHV inoculation. One control pup died 14 days after CHV inoculation and 1 survived until necropsied 19 days after CHV inoculation. Pups given 1/10th dosage of HAI died 8 and 13 days after CHV inoculation. Only one pup given recombinant human alpha-interferon died (12 days after CHV); the other 2 pups survived until necropsied 19 days after CHV inoculation.

These pups, inoculated 3 days after birth, did not develop an overwhelming CHV infection (only 1 of 2 controls died). A 1/10th dose of HAI did not protect either pup but both HAI (1X) treated pups survived.

LITTER 4

Fourteen (14) pups were inoculated orally with CHV 2 days after birth. Seven (7) pups were given PBS and 7 pups were given HAI (1X) orally twice daily for 7 days (if they lived that long) starting 2 days after CHV inoculation. The 7 controls died 1, 5, 7, 8, 8, 9, and 9 days after CHV inoculation. One of the HAI (1X) treated pups survived and the other 6 pups died 1, 6, 8, 9, 9, and 12 days after CHV inoculation. The deaths of 2 pups only 1 day after CHV inoculation were probably not related to CHV inoculation.

One HAI (1X) treated pup lived 3 days beyond the last surviving control and one HAI (1X) treated pup lived 2 weeks (until necropsied) beyond any treated control pup. Average survival time of interferon treated pups was longer than control survival time, but not significantly so.

LITTER 5

Six (6) pups were inoculated orally with CHV 2 days after birth. Three (3) pups were given PBS and 3 were given HAI (1X) orally once daily starting 5 days after CHV inoculation. The 3 controls died 6, 6, and 7 days after CHV inoculation. One of the HAI (1X) treated pups survived (until necropsied) and the others died 8 and 9 days after CHV inoculation.

All interferon treated pups lived longer than any of the control pups. Treatment with HAI (1X) did not begin until 5 days after CHV inoculation, yet survival was significantly ($P<0.05$) prolonged.

In summary, on the average, puppies treated with human alpha-interferon had longer survival times and enhanced survival rates compared to littermate controls, after canine herpesvirus challenge. A total of 7 puppies (1 control and 6 interferon treated) survived the normally fatal CHV inoculation. The data is summarized in the Table 5 below.

TABLE 5

Summary of Canine Herpesvirus Data

Litter	No. of Pups	Dosage	Average* Survival Time (Days)	Survivors
1	3	control	6.33	0
1	3	HAI 1X	7.67	0
1	3	HAI 10X	6.67	0
2	2	control	4.5**	0
2	2	HAI 1/10th	8.5	0
2	2	HAI 1X	7.0	0
2	2	HAI 10X	8.0—	0
3	2	control	14.0	1
3	2	HAI 1/10th	10.5	0
3	2	HAI 1X	—	2
3	3	recombinant IFN	12.0	2
4	7	control	6.7	0
4	7	HAI 1X	7.5	1
5	3	control	6.3	0
5	3	HAI 1X	8.5	1

*dead dogs survival time; living puppies not calculated.
**includes one pup living only one day beyond CHV inoculation.

Treatment Of Nasal Solar Dermatitis

Three cases of nasal solar dermatitis (collie nose) cleared after human alpha-interferon treatment of 1 unit/lb body weight orally and topical treatment (a few ml at 20 units/ml).

Treatment of Canine Lupous Erythematosus

Two cases diagnosed as canine lupus erythematosus were cured by human alpha-interferon treatment. A 2 year-old Lhasa apso male had been treated with prednisolone for 1 year for 3 dermatological lesions on the abdomen and prepuce. The flat glistening lesions were continually licked by the dog. Within 1 week of oral human alpha-interferon treatment (1 unit/lb body weight daily for 5 days, then after 1 week, treatment was repeated for 5 days) 2 lesions completely healed and the third lesion was reduced to ½ its original size. Within 4 weeks, the lesions were all completely healed and all therapy ceased. One year later, a skin lesion reappeared but promptly healed after interferon treatment was repeated. The skin lesions have not reappeared in the past 10 months.

A 6 year-old spayed female chihuahua cross had a spider shaped (4 cm by 2 cm approximately) skin lesion on the abdomen. The lesion was flat, glistening and pruritic. Six weeks of prednisolone treatment resulted in complete healing. The following summer, the lesion reappeared and was treated with oral human alpha-interferon at about 1 unit/lb body weight daily for5 days. Within 5 days the lesion was reduced to ½ its original size and completely disappeared within 10 days. The lesion has not reappeared in the past year.

Treatment Of Feline Infectious Peritonitis

Table 6 shows the results of 17 cases of feline infections peritonitis (FIP) as diagnosed by practicing veterinarians. Human alpha-interferon (IFN) treatment resulted in a significantly greater survival rate than treatment with bovine beta-IFN.

TABLE 6

Survival of clinically ill cats diagnosed as FIP

Treatment	No. Cats Treated	Alive	Dead	Survival Rate
Human alpha-IFN	11	10	1	91%
Bovine beta-IFN	6	3	3	50%
Total	17	13	4	76%

Cats given human alpha-IFN had a significantly (P = .0574) greater survival rate than cats given bovine beta-IFN.

Human Treatment With Exogenous Human Lapha-Interferon

Human patients were treated with human alpha-interferon in the therapy of acute rheumatoid arthritis, multiple sclerosis, astham, acne, malignant lymphoma, mesothelioma, and apthous stomatitis. Therapy consisted of oral administration of 0.7 IU per lb. of patient body weight twice daily, once in the morning and once in the evening. None of the patients noted any fever or anorexia associated with the administration of alpha interferon. Interferon was administered in a buffered solution having a concentration such that a single dosage could be administered in a volume of about 1 to about 20 ml of liquid. Each patient generally retained the interferon solution in his mouth for a period of time up to about one minute. After that time the solution was either swallowed or discharged from the patient's mouth.

Two patients suffering from rheumatoid arthritis were treated—a Caucasian male age 44 and a Caucasian female age 44. The male patient was pain free in 7 days, and the female was pain free in 10 days. They were both continued on the oral interferon for 21 days total and have remained asymptomatic.

It has been found that recurrence of a treated arthritic condition can be minimized if treatment in accordance with the present invention is continued over a period of up to about three months.

A 30-year-old Caucasian female nurse afflicted with multiple sclerosis and who had an extensive neurologic workup at City of Hope Hospital in Los Angeles received treatment in accordance with the present invention for 21 days. The patient has had no recurrence of her neurologic symptoms for the past nine months.

A 42-year-old Caucasian male diagnosed to have a malignant lymphoma had completed chemotherapy with dismal results and was considered terminal. He was treated for three weeks with oral interferon. Six months after starting treatment he was released by his oncologist as free of the disease.

An 82-year-old Caucasian female was diagnosed to have mesothelioma. Presently there is no effective treatment for that disease and only a 9-month average survival rate is predicted. During her treatment with human alpha-interferon she had thoracentesis on two occasions for plural effusion. Otherwise, the patient has been active and has survived for 43 months.

A 32-year-old Asian male with apthous stomatitis was treated for two weeks with human alpha-interferon in accordance with the present invention. There has been no recurrence of the ulcers over the last six months since treatment was completed.

BKC is a 29 year-old Caucasian female and KKJ is a 20 year-old Caucasian female. Both are afflicted by acne-like skin blemishes at the time of their monthly menstrual cycle. Oral human alpha-interferon given at about 1 unit/lb of body weight for 3 days prior to the

TABLE 6-continued

Survival of clinically ill cats diagnosed as FIP

Treatment	No. Cats Treated	Alive	Dead	Survival Rate
beta-IFN				
Total	17	13	4	76%

Cats given human alpha-IFN had a significantly (P = .0574) greater survival rate than cats given bovine beta-IFN.

time of their cycle reduces the severity and number of skin blemishes.

Treatment Of Warts In Humans With Bovine Aloha-Interferon

MAH, a 38 year-old Caucasian female, had 7 warts on the middle finger of her right hard. After 9 months duration, medical treatment was sought, and liquid nitrogen was applied by a dermatologist. Only one wart on the finger regressed after treatment. Three warts coalesced to create a large wart area that, over the next year, acquired a roughly 12 millimeter square shape. Oral bovine alpha interferon treatment was started at a dosage of 6 ml daily for 6 consecutive days. The concentration of alpha-interferon was 30 units/ml; it was derived from the nasal secretions of cattle infected with infectious bovine rhinotracheitis virus. All the warts completely regressed within 6 weeks of the first dose of interferon.

Interferon Dosage Formulations

(1) Lozenge

A starch gel-based lozenge containing interferon is prepared by combining 150 grams of sucrose, 550 ml phosphate buffered saline, and 250 grams of a cold-water-soluble starch such as that described in U.S. Pat. No. 4,465,702, heating that mixture with stirring to a temperature of about 75° C., cooling the mixture to about 30° C. and thereafter blending into the paste-like mass 50 ml of phosphate buffered saline PBS containing human alpha interferon at a concentration of 250 IU/ml. The mixture is then formed into multiple portions of about 5 to about 10 grams each which set upon standing under drying conditions to a starch candy gel-like consistency. The lozenges thereby produced can be administered to a patient singly or in combination. The patient is instructed to hold the lozenge in his mouth until it is completely dissolved to release the interferon component for contact with the oral mucosa.

(2) Chewable Vitamin

A chewable vitamin formulation is prepared, for example, according to the description of U.S. Pat. No. 3,857,939 by coating one or more components thereof prior to tableting with an interferon solution in an amount sufficient to provide about 1 to about 1500 units of interferon in each chewable vitamin tablet.

(3) Mouthwash

A mouthwash formulation is prepared in accordance with the present invention by combining 850 0 ml PBS, 100 ml of glycerin, 50 grams of dextrose, and a mixture of 0.3 ml of a flavor oil pre-mixed with 30 ml of a palatable, pharmaceutically acceptable surfactant/dispersant having an HLB from about 15 to about 25 and 50 ml of a PBS solution of interferon (concentration 120 IU/ml). The formulation contains interferon at a concentration of about 120 IU per 20 ml dosage. The patient is asked to hold a 20 ml volume of the mouthwash in his mouth, optionally gargling with the same, for a period of about 15 seconds to about one minute.

(4) Syrup

Interferon is added to a commercial cough syrup formulation in an amount sufficient to provide an interferon containing syrup formulation having about 1 to about 1500 IU of human interferon per tablespoon of syrup.

(5) Effervescent Tablet

A tableting mixture comprising a pharmaceutically acceptable alkali metal carbonate or bicarbonate, an organic acid such as citric acid, human interferon (preferably dispersed on a suitable organic or inorganic carrier therefor) in an amount sufficient to provide a per tablet dose of about 1 to about 1500 units of interferon per dose, and further including suitable tableting excipients such as lubricants and binders, is compressed into a unitary dosage form of interferon. The compressed tablet effervesces upon contact with water to release interferon to the resulting buffered solution. The dosage of interferon is readily available in solution for contact with the oral pharyngeal mucosa of a patient in need of said dosage of interferon.

I claim:

1. An improved method of treatment of infectious disease of viral origin in human, canine and feline species said method consisting essentially of contacting about 0.01 to about 5 IU of interferon/lb of body weight per dose with the oral and pharyngeal mucosa of said species.

2. The method of claim 1 wherein the dosage is about 0.1 to 4.0 IU/lb per day.

3. The method of claim 1 wherein the interferon is selected from alpha-interferon and beta-interferon.

4. The method of claim 1 wherein the interferon is alpha-interferon produced from human leukocytes.

5. The method of claim 1 wherein human alpha-interferon is administered at dosage of about 0.5 to 1.5 IU/lb of body weight per day.

6. The method of claim 5 wherein a human is treated for a viral infection selected from the group consisting of human rhinovirus, herpes simplex I virus, herpes simplex II virus, viral myocarditis and HILV III virus (AIDS).

7. The method of claim 1 wherein a human is treated for warts.

8. The method of claim 1 wherein a feline species is treated for feline leukemia virus or feline infectious peritonitis.

9. The method of claim 3 wherein a canine species is treated for canine parvovirus.

10. The method of claim 1 wherein canine species is treated for canine herpesvirus.

11. The method of claim 1 wherein a human suffering from Acquired Immune Deficiency Syndrome is treated with human alpha-interferon or human beta-interferon.

12. The method of claim 11 wherein about 0.5 to about 1.5 of interferon/lb of body weight in aqueous solution is held in the patient's mouth for a period of time sufficient to allow contact of the interferon with the oral mucosa of said patient.

13. The method of claim 6 wherein the dosage of interferon is divided and administered as a multiple dose daily regimen.

14. The method of claim 11 wherein beta-interferon is administered at a dosage of about 0.5 to about 1.5 IU/lb per day.

* * * * *

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AN 108:4454 CA

TI Toxicity studies of human fibroblast interferon beta (I). Acute and subacute toxicity studies in mice and rats

AU Shibutani, Yasunori; Obata, Masaomi; Hamada, Yoshimasa; Shichi, Shigeo; Ohi, Keiichi; Kaga, Nobuhiko; Yajima, Gompachi

CS Toxicol. Lab., Mochida Pharm. Co., Ltd., Gotemba, 412, Japan

SO Iyakuhin Kenkyu (1987), 18(4), 571-82
CODEN: IYKEDH

DT Journal

LA Japanese

AB In an acute toxicity study, i.v. or oral administration of 1 .times. 107 - 2.5 .times. 108 IU and i.m. administration of 1 .times. 107 - 5 .times. 107 IU of human interferon .beta. (MR 21)/kg caused no death, apparent symptoms, body wt. change or abnormal autopsy findings in mice and rats. I.v., i.m., and oral LD50 values of MR-21 in mice and rats were >2.5 .times. 108, >5 .times. 107, and >2.5 .times. 108 IU/kg, resp. In a subacute toxicity study, MR-21 administered i.v. to rats for 13 wk at 1 .times. 107 - 3 .times. 105 IU/kg/day caused no death or any symptoms attributable to the administration of MR-21. The no-effect dose level of MR-21 was estd. to be 3 .times. 105 IU/kg/day under the conditions of this study.